# GROWTH SENSITIVITIES TO DRUGS OF FUNGAL PHENOTYPES EXPOSED TO DEEP SPACE IRRADIATION

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Abstract: Two yeast species, Saccharomyces cerevisiae Hansen and Rhodotorula rubra (Demme) Lodder, were included as part of the Microbial Ecology Evaluation Device (MEED) mycology studies of Apollo 16. Phenotypic isolates housed in the MEED spaceflight hardware were selected from each of the yeast species for exposures to known antifungal and experimental compounds. Variation to growth sensitivity induced by the test drugs occurred with the spaceflight phenotypes. It is apparent that induced changes of microorganisms in space can cause variation in drug sensitivity.

Introduction: On board the flight of Apollo 16 in April 1972, during the transearth Extra Vehicular Activity (EVA), the Microbial Ecology Evaluation Device (MEED) was attached to the Apollo Command Module hatch television campole for 10 minutes + 7 seconds by Astronaut Thomas K. Mattingly. After computerized manipulation of the Apollo spacecraft module for the MEED deployment, and using the sunsight and image field on the side of the MEED container, the trays containing the fungal types were exposed to select wavelengths of ultraviolet radiation at a $90^{0}$ angle to the sun (Fig 1).

The experimental package allowed for both qualitative and quantitative evaluation of specific space environment parameters on two filamentous fungi, Chaetomium globosum and Trichophyton terrestre, and two yeasts, Rhodotorula rubra and Saccharomyces cerevisiae. As with other forms of microorganisms, exposure to ultraviolet irradiation on earth or in space causes damage to nucleic acids, and therefore, induces mutational effects (Thornburn, 1972).

Materials and Methods: The MEED hardware was constructed with a series of two filters. The neutral density filters and the bandpass filters in combination presented the specific spaceflight energy parameters received by the yeast cells when exposed to the environment of space (Taylor, 1970). For the exposure of 10 minutes, the filter system was designed to have transmission peaks at 254, 280 and 300 nanometers with sufficient half bandwidths so that the energy levels ranged from 10 to $10^{5}$ ergs per cuvette chamber in which the fungal cells were held (Volz, et al., 1974).

Vegetative yeast cells of the two yeast species were housed in 0.05 $cm^{3}$ lexan cuvettes in the MEED spaceflight hardware. A quartz

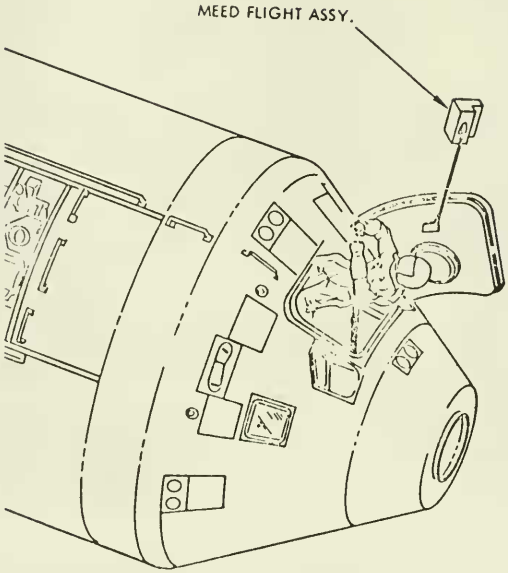


Figure 1. The MEED deployment in space from the Apollo 16 Command Module.

window opposite the fillport allowed the controlled peak wavelengths to enter the cuvette chamber. Cuvettes adjacent to those housing yeast cells contained SO-343 Kodak high resolution film and a potassium ferrioxalate actonometry system for the two methods selected to measure light energy and light intensities entering the cuvettes housing the yeasts. Bimetallic coil spring temperature recorders were equally distributed throughout the MEED hardware trays to monitor the cuvette temperature within the flight case assembly that was insulated to maintain the temperature. Yeast cells were housed in distilled water or dry on filter paper in the cuvettes. Some dry cuvettes were vented to space which did not maintain a 20 C temperature in the cuvette. A limited number of cuvettes received the full light spectrum and intensity of solar irradiation in space that also slightly varied the 20 C temperature maintained in each cuvette.

The paper disk assay method (Iyer and Szybalski, 1958) was used to determine sensitivities of the yeast phenotypes returned from the spaceflight cuvettes. Immediately after splashdown of the Apollo 16 Command Module and the return of the MEED hardware to the NASA Houston laboratory, yeast cells were unloaded from the cuvettes and colonies developed from single cell isolations were grown for phenotype selection (Volz, 1974). A phenotype, for the purpose of the postflight studies, is considered an individually unique entity, based on visual differences between it and the parent or wild type. The variations were caused by exposure to the parameters of space. The wild type of each yeast species was housed in cuvettes retained in the dark on board the flight hardware as well as cuvettes retained in the laboratory subjected to the same temperature controls, no exposures to UV irradiations, and stable atmospheric pressures. The wild types held under various unexposed UV conditions were designated as controls.

Wild types and phenotypes were maintained on Sabouraud's maltose agar (SMA) tube slants at 25 C. Yeast cell suspensions were made in distilled water and 0.1 ml of the suspension was spread evenly over the surface of SMA plates. Drug impregnated disks were placed on the plate aseptically. Drugs selected for the study are listed in Table I. Sterile paper disks were suspended in solutions of the test drugs in concentrations of 10, 100 and 1,000 micrograms per ml. Zones of growth inhibition were read at the end of 7 days incubation at 25 C.

Results: The ability of a test drug to inhibit cellular growth of yeast spaceflight phenotypes was determined by the zones of inhibition. Measurements were made in millimeters from the disk edge to the periphery of the zone exhibiting no cellular growth.

Rate of drug diffusion, drug solubility in addition to the individuality and distinct character of each phenotype regulated the degree of drug sensitivity to each isolate exposed to space-

Table I. Test drugs selected for postflight phenotype studies.

| Drug Code[2] | Mode of Action | Drug Name |
|---|---|---|
| A 1069 | a | 5-Cyclohexylpentyl isothiourea (Sc)[1] |
| A 2545 | g | Dodecamidinium hydrochloride (Sc, Rr) |
| A 3467 | a | bis (2-Hydroxy-5-chlorophenyl) sulfide diethyl amino complex (Sc, Rr) |
| A 4171 | i | 3-Chloro-(4-morpholinopropoxy) biphenyl (Sc, Rr) |
| A 6131 | i | 2-Amino-1,3-dinonyl imidazoline hydrochloride (Sc, Rr) |
| A 9248 | a | Diiodomethyl p-tolyl sulfone (Sc, Rr) |
| A 10811 | a | Diiodomethyl phenyl sulfone (Sc, Rr) |
| A 24007 | c | 2-Cyano-4-nitrothiophene (Sc, Rr) |
| A 30031 | i | 1-Morpholino-2-salicyloylethane hydrochloride (Sc, Rr) |
| A 34320 | a | Benzyl diiodomethyl sulfone (Sc, Rr) |
| A 35980 | a | Cyclohexyl diiodomethyl sulfone (Sc, Rr) |
| A 36033 | a | Diiodomethyl octyl sulfone (Sc, Rr) |
| A 36042 | a | Diiodomethyl 4-methylbenzyl sulfone (Sc, Rr) |
| BW 55079 | e | Polymyxin B (Rr) |
| NSC 185 | e | Actidione (Sc) |
| NSC 9369 | f | 1-Methyl-3-nitro-1-nitrosoguanidine (Sc) |
| NSC 14574 | b | Miracil D (lucanthone hydrochloride) (Sc, Rr) |
| NSC 38583 | i | Carbamic acid, diethyldithio, sodium salt (Sc) |
| NSC 53306 | i | Terephthalanilide-4',4"-bis(2-imidazolin-2-yl-amino) dihydrochloride (Sc, Rr) |
| NSC 84423 | d | Ethidium chloride (Sc, Rr) |
| P 1919 | i | 2-(1'-Oxy-2'-pyridylmercapto) imidazoline hydrochloride (Sc, Rr) |
| P 5351 | h | [Ethyl 4-[β-(o-hydroxybenzoyl)] ethyl]-1-piperazine carboxylate (Sc) |
| P 75082 | e | Nystatin (Sc, Rr) |
| WL 27130 | g | Colistin sulfate (Sc) |
| MK 1145 | e | Chloramphenicol (Sc) |

[1]Drug tested against phenotypes of Saccharomyces cerevisiae (Sc) or Rhodotorula rubra (Rr).

[2]A, Abbott; BW, Burroughs Wellcome; NSC, National Service Center; P, Pfizer, WL, Warner Lambert; MK, McKesson.

flight parameters. Regardless of the factors potentially capable of causing variation, zones of inhibition varied little between phenotypes that received irradiation in space at different energy levels. The phenotypes selected from vegetative cells of R. rubra exposed to full light at energy levels of $10^3$ to $10^5$ ergs per cuvette and at $10^6$ to $10^8$ ergs per cuvette showed constant zones of inhibition within the drug type (Table II). An increase in energy levels in ergs received by the cells in space created no significant change in the average zones of inhibition. Variation did occur with individual phenotypes when other spaceflight parameters were considered.

The cellular response to drugs of spaceflight phenotypes exposed to the same spaceflight parameters or control conditions remained constant yet inhibition rates did vary between phenotypes exposed to different parameters of space. Rhodotorula rubra (Table III) and Saccharomyces cerevisiae (Table IV) phenotypes were selected from different exposures to spaceflight parameters then exposed to the test drugs. Attention was directed to variation between groups of phenotypes and amounts of inhibition with select drug groups. Each number found in the tables is the average of 10 to 25 phenotypes of each test system. In preflight tests a series of experiments were conducted for quality control of the spaceflight hardware (Taylor, 1970). The colonies selected from vibration control studies developed from cells housed in cuvettes in the spaceflight hardware under preflight tests that exposed the hardware to simulated vibrations equal to those of the launch, flight, deployment, and splashdown of Apollo 16. Isolates selected from the vibration control (Table III) exposed to the various drug groups frequently exhibited growth inhibitions similar to ground control isolates. The yeast cells that produced the ground control isolates were contained in cuvettes housed at $20^\circ \pm 5^\circ$ C in the laboratory. Ground control cuvettes were loaded and unloaded at the same time as cuvettes housed in the spaceflight hardware. Full light exposed R. rubra phenotypes in space appeared more sensitive to the drug systems, particularly drug types c, d, and e. Phenotypes exposed to 300 nanometers (nm) light in space were also more sensitive to drug types c and d while other ultraviolet wavelength exposure peaks in space produced no noticable variation in comparison to the various controls.

Saccharomyces cerevisiae cells were not included in full light and vibration control test conditions. The R. rubra test test system was considered sufficient for examining phenotypic changes incurred in space at full light and in the preflight hardware test studies simulating similar stresses in space. Generally significant increases in sensitivity were noted with all phenotypes exposed to space test parameters of 254, 280, 300 nm light, and flight control in spaceflight cuvettes not exposed to light. The flight control phenotypes only received the effects of weightlessness and HZE energy particles that freely penetrated all equipment during the spaceflight (Table IV). Dramatic exceptions to this

Table II. Rhodotorula rubra

Assay disk zones of growth inhibition in mm
at 1,000 mg/ml drug concentration

| Drug type | Energy levels in ergs/cuvette of full light received in space $10^3$-$10^5$ | $10^6$-$10^8$ |
|---|---|---|
| a | 6.07 | 5.97 |
| b | 1.00 | 1.17 |
| c | 8.00 | 8.00 |
| d | 7.20 | 6.00 |
| e | 4.79 | 6.25 |
| f | -* | - |
| g | 2.40 | 2.66 |
| h | - | - |
| i | 3.35 | 2.87 |

-* not tested.
Data represent averages of 10 to 25 phenotypes in replicates of 3 for each test system.

Table III. Rhodotorula rubra

Assay disk zones of growth inhibition in mm
at 1,000 mg/ml drug concentration

| Drug type | Vibration control | Full light | 254 nm | 280 nm | 300 nm | Flight control | Ground control |
|---|---|---|---|---|---|---|---|
| a | 6.62 | 6.52 | 5.91 | 6.67 | 6.27 | 5.21 | 6.37 |
| b | 1.00 | 1.08 | 1.00 | 0.60 | 0.37 | 1.16 | 0.85 |
| c | 5.00 | 8.00 | 5.10 | 5.05 | 7.50 | 5.16 | 5.57 |
| d | 4.16 | 6.67 | 6.10 | 6.72 | 7.66 | 6.66 | 4.92 |
| e | 4.45 | 5.61 | 4.80 | 5.02 | 4.81 | 4.66 | 5.35 |
| f | -* | - | - | - | - | - | - |
| g | 1.66 | 2.53 | 1.75 | 2.93 | 1.75 | 2.60 | 2.50 |
| h | - | - | - | - | - | - | - |
| i | 2.60 | 3.11 | 3.27 | 3.18 | 2.50 | 3.10 | 2.92 |

-* not tested.
Data represent averages of 10 to 25 phenotypes in replicates of 3 for each test system.

Table IV. Saccharomyces cerevisiae

Assay disk zones of growth inhibition in mm
at 1,000 mg/ml drug concentration

| Drug type | Vibration control | Full light | 254 nm | 280 nm | 300 nm | Flight control | Ground control |
|---|---|---|---|---|---|---|---|
| a | -* | - | 9.41 | 9.36 | 9.37 | 8.44 | 8.92 |
| b | - | - | 4.14 | 2.66 | 3.58 | 4.14 | 2.10 |
| c | - | - | 12.00 | 13.06 | 10.35 | 11.11 | 11.81 |
| d | - | - | 17.25 | 12.05 | 12.21 | 13.21 | 4.96 |
| e | - | - | 21.34 | 20.08 | 21.63 | 19.34 | 16.53 |
| f | - | - | 4.00 | 10.50 | 1.00 | 5.66 | 5.33 |
| g | - | - | 6.00 | 6.59 | 2.50 | 9.94 | 8.70 |
| h | - | - | 5.20 | 3.42 | 2.54 | 5.40 | 4.00 |
| i | - | - | 9.44 | 10.54 | 12.01 | 8.00 | 10.33 |

-* not tested.
Data represent averages of 10 to 25 phenotypes in replicates of 3 for each test system.

observation included phenotypes exposed to 300 nm light in space and tested with drug types f, g, and h.

Discussion: A.Interaction of test drugs with cells. Drugs used in the MEED postflight assay can be grouped based on modes of action and chemical composition. Several compounds (a) contain sulfur in its various oxidized forms (Table I). It is interesting to note that fungal resistance is reduced the more the sulfur is oxidized, with sulfides being the most potent, followed by sulfoxides and finally sulfones (Horsfall, Chapman and Rich, 1971). Miracil D (b) complexes strongly, yet reversibly, with DNA and has been shown to inhibit formation of RNA and DNA polymerases (Gale et al., 1972). According to Horsfall (1956), thiophene (c), which is a heterocyclic sulfur compound, is not toxic to fungi based on the fact that it is found within biotin, a vitamin necessary for fungal metabolism. With the addition of the highly toxic cyano group, 2-cyano-4-nitrothiophene does exhibit limited fungal growth inhibition.

The sulfones, sulfoxides and sulfides work along the lines proposed by the Woods - Fildes theory, which states that competitive antagonism exists between p-aminobenzoic acid (the substrate used by microorganisms for normal metabolism) and the sulfur containing derivatives. When the test drugs are bound to the yeast cells, metabolic activities and enzyme actions normally carried on are permanently blocked or disrupted resulting in various degrees of growth inhibition (Goodman and Gilman, 1972).

Ethidium chloride (d) is another drug that interferes with the synthesis of nucleic acids. It forms reversible, but quite strong, non-covalent bonds with DNA, and a high degree of interference of RNA polymerase actions. Ethidium chloride has also been found to have a deleterious effect on the mitochondria of eucaryotic cells. A drug with this mode of action is termed an intercalating drug (Gale et al., 1972).

Actidione, or cycloheximide, is one of a group of polyene antibiotics (e) which is found to be effective in suppression of ribosomal activities in so far as they relate to the synthesis of proteins (Gottlieb and Shaw, 1967). Actidione was tested only against phenotypes of S. cerevisiae because preflight testing had shown that this yeast normally is not resistant to this drug (Volz, 1974). It is important to note that polyene compounds have not been shown to inhibit enzymes and their systems in vitro (Burger, 1970). In a reversible reaction, cycloheximide affects some of the transfer functions important in the biosynthesis of proteins.

Another polyene antibiotic tested only against Saccharomyces phenotypes was found to be the most potent in terms of drug sensitivity reactions and inhibition zone sizes. This drug, chloramphenicol, both interferes and inhibits the synthesis of proteins needed for the metabolism of a varied group of microbial organisms

(Physician's Desk Reference, 1974).

Nystatin, which falls into the classification of polyene antibiotics, also was found to be an extremely effective drug in these studies. Its main mode of action is to adversely affect the permeability of sensitive cell membranes. In addition, any cell whose membrane contains steroid compounds is very susceptible to nystatin's effect (Gale, et al., 1972). When cell membrane permeability is altered, leakage of potassium ions, inorganic phosphate, phosphate esters and carboxyl and amino acids from within the cell results in and rapidly causes the destruction of that cell. This "detergent" effect is highly specific for yeasts, as both Rhodotorula and Saccharomyces species contain small quantities of steroid substances. Microorganisms lacking sterols in their membranes have all been found to be unaffected by nystatin (Goodman and Gilman, 1972). Polymyxin B, a polyene antibiotic, has a mechanism of action similar to that of nystatin. The drug was tested only against R. rubra phenotypes, but it was not found to be a very effective inhibitor.

1-Methyl-3-nitro-1-nitrosoguanidine (f), a powerful mutagen that is used as an antileukemic compound in vivo and exhibits potent antifungal properties in vitro, disturbs the functioning and intercellular synthesis of both nucleic acids and proteins. In this way, the mode of action of this drug is somewhat comparable to those of the polyene antibiotics. The primary function is fungistatic (Goodman and Gilman, 1972).

Two other test drugs selected for use in the MEED mycology studies are surface active agents, or cell surfactants (Gale, et al., 1972). Colistin sulfate and dodecamidinium hydrochloride are microbiocidal in action (g) with the former drug effective against gram negative organisms only. Similar in mode of action to the polyene compounds, the cell membrane permeability is altered and results in the dessication of the cell and the leakage of vital cellular constituents. Primarily used as an antihelmintic compound against Enterobius vermicularis and Ascaris lumbricoides, [ethyl 4-[β-(o-hydroxylbenzoyl)] ethyl]-1-piperazine carboxylate (h) was found to have inhibitory effects against most returned phenotypes of S. cerevisiae. With Ascaris this compound was found to have an effect on cell membrane permeability, thereby resulting in leakage, dehydration, and death (Goodman and Gilman, 1972).

Little is known or current studies are underway on additional chemicals (i) selected for the MEED studies. Some of these drugs were recently synthesized and serve only as experimental compounds presently undergoing assays for potency and toxicity. Most of these drugs were moderate in their inhibition of returned space-flight phenotypes and controls (flight, ground and vibration). Chemicals in this group include 3-chloro-(4-morpholinopropoxy) biphenyl; 2-amino-1,3-dinonyl imidazoline hydrochloride; 1-morpho-

lino-2-salicyloylethane hydrochloride; carbamic acid, diethyldithio, sodium salt; terephthalanilide-4',4"-bis(2-imidazolin-2-yl-amino) dihydrochloride; and 2-(1'-oxy-2'-pyridylmercapto) imidazoline hydrochloride.

B. Stresses upon yeast cells housed in the MEED cuvettes. Radiation damage caused by direct and indirect ionization probably was the cause of changes incurred by yeast cells exposed in the experimental cuvettes (Thornburn, 1972; Lawrence and Block, 1968). Damage probably was caused by the primary ionization of water housing the spaceflight cells in suspension in the cuvettes, followed by radiolysis reactions leading to hydrogen gas and hydrogen peroxide end products (Thornburn, 1972). Direct ionization of cells could also cause damage particularly to cells housed dry in the cuvettes containing no water (Thornburn, 1972; Fedorova, 1964).

Induced changes in both experimental and control groups gave rise to unique phenotypic entities, each with their own characteristic reaction to a given set of stimuli (test drugs) utilized in this study. Reannealing of injuries occur at the molecular level in UV studies of dark photoreactivation (Thornburn, 1972; Fedorova, 1964; Silverman and Davis, 1964). This phenomenon could have occurred at the individual cell level during transit to the NASA Houston Lunar Receiving Laboratory. Each recovered phenotype was a visually identified distinct and separate variation produced from the original wild type as a result of changes elicited by UV irradiation or possibly other stress factors incurred by one particular cell.

Other stress factors that might possibly have induced the yeast cells to change were encountered by flight organisms. Some of these same factors were probably responsible for slight changes noted among the control groups on the ground. The MEED box was a basically closed environmental system. Temperature was maintained at approximately 25 C and did not fluctuate during the short 10 minute spaceflight exposure. This is important to consider for cells housed dry were vented to space. It has been shown that the viability of microorganisms is preserved at temperatures close to absolute zero (Imshenetsky, 1963). Also, the sub-atmospheric pressure, more accurately termed a "vacuum", could be a pertinent and important stress encountered by organisms in the selected cuvettes housing cells dry and exposed to the space atmosphere. Weightlessness is also a factor that may have played a role in inducing changes in flight experimental and control groups, as is the reduced $O_2$ tension and the presence of the high energy multicharged particles (HZE) that penetrate everywhere in space except for earth's protective atmosphere (Bourne, 1963; Silverman and Davis, 1964; Imshenetsky and Lysenko, 1965).

An environmental condition, abnormal to the yeast species as existed in cuvettes, regardless of where they were during the flight, could also cause cell stress at the individual cell level. While

held in this abnormal state, it is no doubt likely that a buildup of metabolic wastes within some of the cells to toxic levels could have occurred. Quite possibly other stress factors intervened to slow yeast cell metabolism. Other environmental stress factors to consider are abnormal fermentation and respiration processes (which no doubt were held in check because of lack of proper substrate) and effects of mechanical stimulations, including vibration and rapid acceleration. All of the above factors may have played an important role in causing the observed changes in experimental groups. However, variation to drug sensitivity did occur with the phenotypes that were exposed to selected spaceflight parameters.

C. Drug actions and the relevance of the selected assay system. Those drugs used against the isolated yeast phenotypes of Saccharomyces cerevisiae and Rhodotorula rubra fall into several distinct categories in regards to their mechanism of action. Ethidium chloride and Miracil D act on the molecular level, causing disruption of normal replication, transcription and translation process (Gale, et al., 1972). Drugs such as actidione and chloramphenicol utilized in this study were found to have a deleterious effect on ribosomal activities (Gale, et al., 1972). Another drug group are those agents which affect the intermediary metabolism of microorganisms. They also cause disruption or inhibition of key enzymes in vitro. Examples of these compounds are sulfones, sulfides and other sulfur containing drugs such as 2-cyano-4-nitrothiophene (Goodman and Gilman, 1972; Gottlieb and Shaw, 1967). The final group of drugs that can be listed are those that act on the cell membrane, causing a "detergent" effect in altering the permeability of the cell membrane, and therefore causing the leakage of important cellular metabolites including inorganic phosphate, phosphate esters and electrolytes (i.e., $K^+$, $Ca^+$) outside the cell. Cells rapidly desiccate and finally die (Gottlieb and Shaw, 1967; Goodman and Gilman, 1972). This category of action can be further subdivided into those drugs that disrupt and cause faulty synthesis of cell membrane structure or that disrupt membrane proteins that deal with intracellular transport processes. Dodecamidinium hydrochloride has these capabilities. In addition, other compounds exist which can cause specific and selective changes in the permeability of cell membrane walls of those microorganisms containing small amounts of sterols. The yeasts Saccharomyces cerevisiae and Rhodotorula rubra are reported to contain wall sterols (Lodder, 1970; Burger, 1970). A well known drug included in the latter category is nystatin (Goodman and Gilman, 1972; Gottlieb and Shaw, 1967). Both Saccharomyces and Rhodotorula isolates seemed to be more sensitive to nucleic acid and protein inhibitors, surface active agents (cell membrane disruptors) and intermediate metabolism inhibitors (the sulfones and sulfides). The latter compounds compete with the normal microbial substrate (Goodman and Gilman, 1972; Gottlieb and Shaw, 1967). Those drugs that tend to be least effective are among those whose action is as yet unknown. One of the drugs with a known mode of action (disturbs functioning

of synthesis of proteins and nucleic acids) that was not effective at inhibiting Saccharomyces phenotypes was 1-methyl-3-nitro-1-nitrosoguanidine. This is a powerful mutagen used as an anti-leukemic agent in vivo (Goodman and Gilman, 1972).

The assay method of filter paper disks saturated with the test drug employed in this MEED Apollo postflight study is an adaptation of the Kirby - Bauer single disk zone assay (Harris and Coleman, 1963; Gradwohl, 1956; Difco Manual, 1971). The relative reproducibility, uniformity, rapidity and ease of application to this unique study led to its selection for postflight analysis of isolated phenotypes over other assay methods presented in the literature. Another such method for antibiotic testing is the tube method, in which serial dilutions of the test agent is made in culture media inoculated with a quantitative amount of a stock test organism (Difco Manual, 1971). The end point determination or the effective range of antibiotic potency is identified where growth is inhibited by the lowest dilution of the drug. This method requires exact concentrations of drug solutions in order for the test results to be valid. Since some of the test drugs in this study were not completely soluble in water, and the exact concentration per milliliter would not be known, the tube method was not selected for this assay. This method also is more commonly used in testing the effectiveness of antiseptics, disinfectants and liquid sterilizing solutions such as phenol (Goodman and Gilman, 1972; Difco Manual, 1971; Lawrence and Block, 1968).

Summary: As a result of exposure to UV radiation and abnormal environmental and physical stresses, changes were induced in the cells of Saccharomyces cerevisiae and Rhodotorula rubra housed in the Apollo 16 spaceflight hardware. Unique yeast phenotypes were isolated for these studies as separate and distinct entities from the original wild type and from each other. Distinctions were based on such factors as colony morphology, colony texture, colony growth rate and differences in colony pigmentation. These individuals were then tested against test drugs to determine growth sensitivities, variations and similarities. A modification of the Kirby-Bauer single disk assay was utilized to determine drug inhibitions. Drug sensitivities and sizes of the zones of inhibition were measured and tabulated.

Results indicate that there were distinct variations among tested phenotypes. In general, cells exposed to spaceflight parameters produced phenotypes more sensitive to test drugs. Exposure to various energy levels at a given wavelength in space apparently was insignificant for variation in drug sensitivity. Each phenotype isolated for postflight evaluation reacted as an individual entity, a descendant from a wild type, yet not looking or reacting in a way similar to the parent. It seems clear that phenotypic change occurred within the cell at the molecular level and that a changed genotype was responsible for giving rise to each individual phenotype. Other previously published evidence on the Apollo 16 MEED mycology

test organisms supports this theory (Volz, 1973; 1974;1975; Volz and Dublin, 1973; Volz, et al., 1974; Dublin, et al., 1974; Sawyer, et al., 1975).

Acknowledgments: The authors greatly appreciate the assistance of R. C. Simmonds, J. L. Hiser, D. E. Jerger and J. M. Veselenak during these studies at the Lunar Receiving Laboratory, NASA Johnson Space Center, Houston. Abbott Laboratories, North Chicago, Illinois; Burroughs - Welcome & Co. (U.S.A.) Inc., Research Triangle Park, North Carolina; McKesson Laboratories, Bridgeport, Connecticut; Pfizer Pharmaceuticals, Groton, Connecticut; Warner Lambert Research Institute, Morris Plains, New Jersey; and the Department of Health, Education and Welfare Public Health Service, Washington, D.C. kindly furnished the test drugs for these studies.

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